



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 10, 2008

MEMORANDUM

SUBJECT: Review of Leach Rate Data for SeaGuard® HMF 1 Antifouling Paint - Red (Alternate Formulation) Containing Ecomea (7.28%) and Zinc Pyrithione (6.38%) Antifouling Agents, EPA File Symbol 577-LTN

FROM: Srinivas Gowda, Microbiologist/Chemist *Srinivas Gowda*
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TO: Marshall Swindell, Team Leader, Team 33
Demson Fuller, Risk Manager Reviewer
Regulatory Management Branch I
Antimicrobials Division (7510P)

THRU: Siroos Mostaghimi, Team Leader, Team one *Siroos Mostaghimi*
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Norman Cook, Branch Chief *Norman Cook*
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

DP Barcodes: D345726

Decision #: 384285

Case Type: New Registration

Common Name: Ecomea 028

PC Code: 119093

CAS#: 122454-29-9

Chemical Name:

Chlorophenyl-cyano-bromo-trifluoro-methyl pyrrole

EPA File Symbol: 577-LTN

MRID No.: 472389-12

Data Submitter: Sherwin-Williams Company

Common Name: Zinc Pyrithione (Zn Omadine)

PC Code: 088002

CAS#: 13463-41-7

Chemical Name: Zinc 2-Pyridinethiol 1-Oxide

INTRODUCTION:

Sherwin-Williams Company, has submitted the Special Leaching Study for SeaGuard HMF Antifouling Paint - Red, containing 7.28% Ecomea 028 (PC Code 119093) and 6.38 % Zinc Pyrithione (PC Code 088002) antifouling agents to meet the U.S. Environmental Protection Agency's Environmental Fate Data Requirements in support of the alternate formulation, HMF Antifouling Paint-Red, EPA File Symbol 577-LTN. The submitted Special Leaching Study has undergone review by Srinivas Gowda of Antimicrobials Division's Risk Assessment and Science Support Branch. The registrant used the ASTM Standard Test Methods D 5108-90, D 6442-05, and D1141-98 that are acceptable by the Agency to fulfill the Special Leaching Study Data Requirements for Antifouling Paints.

BACKGROUND:

Ecomea 028 (PC Code 119093) and Zinc Pyrithione (PC Code 088002) are active ingredients in SeaGuard HMF Antifouling Paint-Red , EPA File Symbol 577-LTN. The submitted study was conducted to determine the leaching rate of Ecomea 028 (CAS Number: 122454-29-9) and Zinc Pyrithione (CAS No. 13463-41-7) from a painted surface exposed to synthetic seawater.

CITATION:

Determination of the Leach Rate of Zinc Pyrithione and Ecomea® 028 from Seaguard HMF 1 in Substitute Ocean Water by Kelda Serak, ABC Laboratories, Inc., 7200 E. ABS Lane, Columbia Missouri 65202 , ABC Study No. 49912, ABC Report No. 49912-1, dated August 28, 2007 (MRID No. 472389-12).

DATA EVALUATION REPORT: Attached.

CONCLUSIONS:

Zinc pyrithione:

Maximum average leach rate:	24.1 µg/cm ² /day (Day 1).
Minimum average leach rate:	5.63 µg/cm ² /day (Day 84).
Cumulative leach rate:	884 µg/cm ² (Day 0-91).
Mean leach rate from day 21 to day 91:	8.27 µg/cm ² /day.

Ecomea® 028:

Maximum average leach rate:	13.5 µg/cm ² /day (Day 31).
Minimum average leach rate:	6.76 µg/cm ² /day (Day 77).
Cumulative leach rate:	912 µg/cm ² (Day 0-91).
Mean leach rate from day 21 to day 91:	10.1 µg/cm ² /day.

RECOMMENDATIONS:

The submitted Special Leaching Study is acceptable and reflects the guideline specified for the ASTM Standard Test Method, D6442-06 for aqueous availability. Risk Assessment and Science Support Branch recommends that the Special Leaching Study for the Ecomea 028 (PC Code: 119093; CAS Number: 122454-29-9) and Zinc Pyrithione (PC Code 088002; CAS No. 13463-41-7) antifouling agents be accepted in support of the alternate formulation, HMF Antifouling Paint-Red, (EPA File Symbol 577-LTN) End-Use Product registration.

**DETERMINATION OF THE LEACH RATE OF ZINC PYRITHIONE AND ECONEA
028 FROM SEAGUARD HMF 1 IN SUBSTITUTE OCEAN WATER**

DATA EVALUATION RECORD

PRODUCT FORMULATION: SeaGuard HMF 1 Antifouling Paint Red.

ACTIVE INGREDIENTS:

Zinc Pyrithione:	Bis(1-hydroxy-2(1H)-pyridinethionato-o,s)-T-4)zinc.
Econea® 028:	2-(p-Chlorophenyl)-3-cyano-4-bromo-5-trifluoromethyl pyrrole.

BACKGROUND:

The submitted leaching study was conducted to determine the leach rate of zinc pyrithione and Econea® 028 from Seaguard HMF 1 paint in substitute ocean water. For a biocide, one major route of entry into an aquatic environment is the leaching from the surface of a vessel. The data obtained from this study are intended for use in modeling that predicts environmental concentration and assesses environmental risk.

CITATION:

Study Title:	Determination of the leach rate of organic biocides from two paints in substitute ocean water: Seaguard HMF 1 and Seaguard HMF 2.
Report Title:	Determination of the leach rate of zinc pyrithione and Econea® 028 from Seaguard HMF 1 in substitute ocean water.
Study Author:	K. Serak.
Date:	August 28, 2007.
Performing Laboratory:	ABC Laboratories, Inc., 7200 E. ABS Lane, Columbia, Missouri 65202.
Study Sponsor:	The Sherwin-Williams Company, 101 West Prospect Avenue, Cleveland, Ohio 44115-1075.
Study No.:	ABC Study No.: 49912; ABC Report No.: 49912-1.
MRID No.:	47238912.
GUIDELINE Nos.:	ASTM D 5108-90 and ASTM D 6442-05.

EXECUTIVE SUMMARY:

The study was conducted to determine the leach rate of zinc pyrithione and Ecomea® 028 from Seaguard HMF 1 Paint Red in substitute ocean water under laboratory conditions over a period of 91 days. The study was conducted according to ASTM methods D 5108-90 and D 6442-05, and in compliance with USEPA 40 CFR Part 160. The study report was reviewed to be in accordance with ASTM Designation: D6442-06. Three test polycarbonate cylinders (*ca.* 17.5 cm length; 6.3 cm outside diameter) per treatment were masked with a 200 cm² area of formulated test paint (duplicate coats), dried for 7 days and suspended in a sample holding tank held within a dissolution water bath maintained at 25°C, pH 7.9-8.1 and salinity 33-34 ppt. The pH was adjusted using 0.1N NaOH and/or 0.1M HCl. Water was pumped from the outlet and either entered a fluidized bed filter or entered two activated carbon filters and a particle filter. The water was continuously circulated through the holding tanks at a rate of 2-8 turnovers per hour. The water exited the holding tank through tubing set to an “overflow” height. The entire system was held in a water bath maintained at 25 ± 2°C. One control polycarbonate cylinder per treatment was also prepared. Measurements of water temperature, pH, salinity and zinc pyrithione and Ecomea® 028 content were conducted at least at every sampling interval.

The paint cylinders were sampled on days 1, 3, 7, 10, 14, 21, 24, 28, 31, 35, 38, 42, 45, 51, 58, 63, 70, 77, 84, and 91. Saltwater was added to each of three labelled sample collection vessels placed into the waterbath of the dissolution system. Three paint cylinders for each treatment were removed from the holding tanks, attached to the sample collection stirrer, spun for 1 hour at 60 rpm, then removed and placed back into their respective holding tank. The control sample was also sampled as described. After 91 days, the paint cylinders were removed, rinsed with water, allowed to dry, and the thickness of the remaining paint was measured with a micrometer.

The study was conducted under laboratory conditions using synthetic ocean water. The average temperature of the system was 25.3 ± 0.1°C throughout the study. The turnover rates of the chambers ranged from 5.11 to 6.38 per hour. The pH of the system ranged from 7.86-8.08, and the salinity ranged from 30.4-35.1 ppt. The thickness of the paint on the cylinders prior to study initiation was >100 µm. At study termination, the thickness was >50 µm (see Reviewer’s Comment).

The synthetic ocean water was analyzed for zinc pyrithione using HPLC. Zinc pyrithione standards were prepared in order to generate a calibration curve. A spike of zinc pyrithione was also prepared to validate the HPLC recovery. The synthetic ocean water samples, reference standards, calibration standards, and spike were first treated with 2,2-dithiodipyridine solution and EDTA solution. Zinc pyrithione was identified by comparison to chromatograms of a derivatized reference standard. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were 1.41 ng/mL and 4.5 ng/mL, respectively. Once per week, the water in the system was tested for the presence of zinc pyrithione by analyzing aliquots of the water from between the two carbon filters.

The synthetic ocean water was analyzed for CL 322,250, a transformation product of Ecomea® 028, using LC-MS/MS. Ecomea® 028 standards were prepared in order to generate a calibration curve. A spike of Ecomea® 028 was also prepared to validate LC-MS/MS. The reference

standards, calibration standards, and spike were first treated with 2,2-dithiodipyridine solution and EDTA solution. Prior to analysis, the synthetic ocean water samples were allowed to sit overnight at room temperature to allow the Ecomea® 028 to hydrolyze naturally to its transformation product CL 322,250. The LOD and LOQ were 0.157 ng/mL and 0.5 ng/mL, respectively. Once per week, the water in the system was tested for the presence of Ecomea® 028 by analyzing aliquots of the water from between the two carbon filters.

For **Zinc pyrithione**: The mean release rate from the painted cylinders from Day 21 to Day 91 was 8.27 $\mu\text{g}/\text{cm}^2/\text{day}$. The cumulative release rate from the painted cylinders was 884 $\mu\text{g}/\text{cm}^2$ at Day 91. The release rate reached a pseudo-steady state beginning with Day 58. The mean release rate over the pseudo-steady state period (Day 58 to Day 91) was 6.12 $\mu\text{g}/\text{cm}^2$. The calibration curve which was generated from the series of zinc pyrithione standards yielded correlation coefficients of ≥ 0.995 . Spike recovery ranged from 92.2-103.5%. Zinc pyrithione was never detected in the re-circulation tank and system in the weekly test for breakthrough.

For **Ecomea® 028**: The mean release rate from the painted cylinders from Day 21 to Day 91 was 10.1 $\mu\text{g}/\text{cm}^2/\text{day}$. The cumulative release rate from the painted cylinders was 912 $\mu\text{g}/\text{cm}^2$ at Day 91. The release rate did not reach a pseudo-steady state. The calibration curve which was generated from the series of zinc pyrithione standards yielded correlation coefficients of ≥ 0.995 . Spike recovery ranged from 90.1-106.5%. The CL 322,250 standards had recoveries ranging from 95.5-105.4%. Neither Ecomea® 028 nor CL 322,250 was ever detected in the re-circulation tank and system in the weekly test for breakthrough.

This study is scientifically sound and classified as acceptable. No significant deviations from good scientific practices were noted. The following deviations from ASTM Designation D6442-06 were noted: the exposure surfaces of the test cylinders were prepared by lightly sanding with 400 grit sandpaper prior to application of the test paint; the cleaning/treatment of the laboratory ware prior to use in the study was not reported; the relative humidity during the 7-day drying period of the painted cylinders was not reported; and it was not reported whether the test cylinders were rotated during the study.

RESULTS SYNOPSIS:

Zinc pyrithione:

Maximum average leach rate:	24.1 $\mu\text{g}/\text{cm}^2/\text{day}$ (Day 1).
Minimum average leach rate:	5.63 $\mu\text{g}/\text{cm}^2/\text{day}$ (Day 84).
Cumulative leach rate:	884 $\mu\text{g}/\text{cm}^2$ (Day 0-91).
Mean leach rate from day 21 to day 91:	8.27 $\mu\text{g}/\text{cm}^2/\text{day}$.

Ecomea® 028:

Maximum average leach rate:	13.5 $\mu\text{g}/\text{cm}^2/\text{day}$ (Day 31).
Minimum average leach rate:	6.76 $\mu\text{g}/\text{cm}^2/\text{day}$ (Day 77).
Cumulative leach rate:	912 $\mu\text{g}/\text{cm}^2$ (Day 0-91).
Mean leach rate from day 21 to day 91:	10.1 $\mu\text{g}/\text{cm}^2/\text{day}$.

I. MATERIALS AND METHODS

Guideline followed: The study was conducted according to ASTM methods D 5108-90 and D 6442-05 (p. 10).

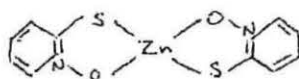
Compliance: The study was conducted in compliance with USEPA 40 CFR Part 160 (p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A Certificate of Authenticity was not provided; however, a signature page was included (p. 6).

A. Materials:

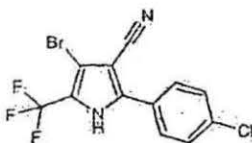
1. Test Material: SeaGuard HMF 1 Paint Red (Sherwin Williams Company, Chicago, Illinois; Batch No. 2004-232-184; p. 11).

Active ingredients: Zinc pyrithione and Econea® 028 (p. 10).

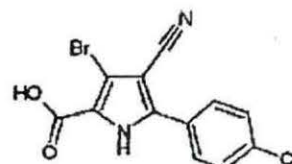
Chemical Structures: Appendix 1, pp. 59-62.



Zinc pyrithione



Econea 028



CL 322,250

Description: Zinc pyrithione: white powder.

Purity: Not reported.

Physical and Chemical properties of Econea 028 Technical:

Appearance	off-white to light brown powder	Solubility
Odour	slightly sweet, marzipan like	Water solubility
pH	5.16 in a saturated solution	0,16 mg/L
Molecular weight	349.5	Fat solubility
Boiling point	()	()
Melting point	onset 252.3°C	Solubility in solvents (at 20 °C)
Vapour pressure	1,9 x 10 ⁻⁴ Pa at 20°C	Acetone
Bulk density	0.45 kg/l	300.5 g/L
Pour density	0.521 g/mL	Ethyl Acetate
Tap density	0.647 g/mL	236.0 g/L
Relative density	1.714 g/ml at 20°C	n-Heptane
Decomposition temperature	247 °C	7.2 g/L
		Methanol
		109.1 g/L
		n-Octanol
		85.2 g/L
		Xylene
		5.6 g/L
		Other data:
		Miscibility
		()
		Evaporation rate
		()
		Conductivity
		()
		pK/a
		7.08
		Refraction index
		()

Physical and Chemical properties of Zinc Omadine Powder Technical:

Physical State:	powder
Color:	off-white to tan
Odor	None
Molecular Weight:	(Active ingredient)317.68
pH	6.5 - 9 (5% solution in neutral, distilled water)
Octanol/Water Coeff:	No data
Solubility in Water:	8 ppm
Viscosity:	Not applicable
Bulk Density:	0.35 g/cc
Specific Gravity:	1.78
Vapor Density:	Not applicable
Evaporation Rate:	Not applicable
Boiling Point:	No data
Melting Point:	240 Deg. C. / 464 Deg. F.
Freezing Point:	No data
Volatiles, % by vol.:	No data
VOC Content %w/w / lbs/gal:	0.00 / 0.00
HAP Content %w/w / lbs/gal:	0.00 / 0.00

Series 830 Physical/Chemical properties of HMF Antifouling paint (End-Use Product)

14. GUIDELINE REFERENCE NO.(GRN)/TITLE	15. VALUE or QUALITATIVE DESCRIPTION/METHOD(s) USED WHERE APPLICABLE AND REFERENCES
-6302 Color	Black color
-6303 Physical State	Liquid
-6304 Odor	Paint Odor
-6314 Oxidation/Reduction: Chemical Incompatibility	Compatible with Water, 10% Monoammonium phosphate, Iron Powder, 10% Potassium Permanganate, and Kerosene
-6315 Flammability/Flame Extension	39.2 +/- degrees C
-6316 Explodability	Data Waiver requested. See Volume 6.
-6317 Storage Stability	6-month report available. The product is stable at 6 months. One year data point will be collected by January 10, 2008.
-6319 Miscibility	Data Waiver requested. See Volume 6.
-6320 Corrosion Characteristics	6-month report available. The product container showed no changes at 6 months. One year data point will be collected by January 10, 2008.
-6321 Dielectric Breakdown Voltage	Data Waiver requested. See Volume 6.
-7000 pH	6.33 +/- 0.04
-7100 Viscosity	2548.854 centistokes at 20 C & 720.589 centistokes at 40 C
-7300 Density/Relative Density/ Bulk Density	Density @ 20 C 1.493 g/mL

EXPLODABILITY -- OPPTS 830.6316

Product does not contain any ingredients that are explodable.

MISCIBILITY -- OPPTS 830.6319

Product is not waterborne. It is a petroleum solvent borne coating, and thus completely soluble in petroleum solvents.

Dielectric Breakdown Voltage -- OPPTS 830.6321

Not applicable. Not to be used around electrical equipment.

Parameter	Zinc pyrithione	Econea® 028	CL 322,250
Molecular weight	317.7 g/mole.	349.5 g/mole.	325.55 g/mole.
Molecular formula	C ₁₀ H ₈ N ₂ O ₂ S ₂ Zn	C ₁₂ H ₅ BrClF ₃ N ₂	C ₁₂ H ₆ BrClN ₂ O ₂

Data were obtained from pp. 10-11 and Appendix I, pp. 59-62 of the study report.

2. Reference Standards: Zinc pyrithione (p. 10).
Econea® 028.
CL 322,250 (p. 11).

Chemical Structure: See DER Attachment 1.

Description: Zinc pyrithione: white powder (Appendix I, p. 59).

Purity:

Zinc pyrithione: Analytical purity: 98.3% (p. 10).
Reference No. PS-18276.
Lot No.: 0108244691.

Econea® 028: Analytical purity: 99.3% (p. 10).
Reference No. PS-18389.
Lot No.: AC11535-95A.

CL 322,250: Analytical purity: 92.6% (p. 11; Appendix I, p. 62).
Reference No. PS-18482.
Lot No.: AC12395-43.

3. Synthetic Sea Water Solution:

Synthetic ocean water (saltwater) was prepared by adding NaCl and Na₂SO₄ to reagent water in a carboy (p. 13). Volumes of Stock Solution 1 (prepared by adding 2200 g of MgCl₂ x 6H₂O, 230 g of CaCl₂, and 9 g of SrCl₂ x 6H₂O in 4 L of reagent water) and Stock Solution 2 (prepared by adding 280 g of KCl, 40 g of KBr, 1 g of NaF, 80 g of NaHCO₃, and 11 g of H₃BO₃ in 4 L of reagent water) were added and the solution was brought to volume with reagent water. The saltwater was prepared according to ASTM Method D 1141-98. If necessary, the pH was adjusted to 7.9-8.1 using 0.1N NaOH and/or 0.1M HCl and the salinity was adjusted to 33-34 ppt with reagent water and/or 5M NaCl. The saltwater was stored in an environmental chamber at 25 ± 2°C for a minimum of 48 hours.

B. Experimental Conditions:

1. Preliminary Study:

Preliminary experiments were not conducted.

2. Calibration and Standardization:

Two stock solutions of zinc pyriithione were prepared four times during the study at a nominal concentration of *ca.* 500 µg/mL with dimethyl sulfoxide (p. 11). For each analysis, aliquots from one stock solution were diluted to 10 mL with reagent water for use as calibration standards and an aliquot from the other stock solution was diluted with reagent water for use as a spiking solution.

A solution of Ecomea® 028, designated CDG 4472, was prepared at a nominal concentration of 524 µg/mL by weighing Ecomea® 028 (26.4 mg) into a 50 mL flask and bringing to volume with acetonitrile (p. 11). For use as a spiking solution and for the definitive tests, the solution was diluted by transferring an aliquot (0.5 mL) to a 50 mL flask and bringing to volume with acetonitrile.

A solution of CL 322,250, designated CDG 4474, was prepared at a nominal concentration of 500 µg/mL by weighing CL 322,250 (27 mg) into a 50 mL flask and bringing to volume with tetrahydrofuran (p. 11). For use as calibration standards and for the definitive tests, the solution was diluted by transferring an aliquot (1 mL) to a 50 mL flask and bringing to volume with acetonitrile. The solution was further diluted by transferring an aliquot (2.5 mL) to a 50 mL flask and bringing to volume with acetonitrile:water (50:50, v:v). Volumes of this solution were diluted further with acetonitrile:water (50:50, v:v); further details were not provided.

A solution of CL 322,250, designated CDG 4475, was prepared at a nominal concentration of 504 µg/mL by weighing CL 322,250 (27.2 mg) into a 50 mL flask and bringing to volume with tetrahydrofuran (p. 12). For use as a spiking solution, the solution was diluted by transferring an aliquot (0.5 mL) to a 50 mL flask and bringing to volume with acetonitrile. The diluted solution was further diluted by transferring an aliquot (5 mL) to a 50 mL flask and bringing to volume with acetonitrile:water (50:50, v:v). This solution was further diluted by transferring an aliquot

(2 mL) to a 50 mL flask and bringing to volume with acetonitrile:water (50:50, v:v). This solution was used as an HPLC QC check standard.

3. Experimental Conditions:

Polycarbonate cylinders (*ca.* 17.5 cm length; 6.3 cm outside diameter; 3 test cylinders, one control cylinder per treatment) were sealed on one end with a piece of polycarbonate using methylene chloride (pp. 14-15, 19). A hole was drilled *ca.* 0.5 cm from the open end for attaching each paint cylinder to the sample collection system, which consisted of 2 L glass beakers containing 3 glass rods (*ca.* 6 mm i.d.) to serve as baffles (p. 15; Figure 1, p. 40). The cylinders were prepared by lightly sanding with 400 grit sandpaper and wiping with a Kimwipe (pp. 16-17). Each test cylinder was masked with a 200 cm² area of test paint, mixed with o-xylene (90:10, v:v), using a power sprayer and allowed to air dry. After a second application of the test paint, the cylinders were allowed to dry at room temperature for 7 days. The paint thickness was measured with a micrometer. The cylinders were then lowered into holding tanks that were held within a dissolution water bath maintained at 25°C (p. 15; Figure 2, p. 41). Water was pumped from the outlet where about half entered a fluidized bed filter and the other half entered two activated carbon filters and a particle filter. The water was continuously circulated through the holding tanks at a rate of 2-8 turnovers per hour. The water exited the holding tank through tubing set to an “overflow” height (p. 16). The entire system was held in a water bath maintained at 25 ± 2°C. The water was measured daily for pH (7.9-8.1) and salinity levels (33-34 ppt); if necessary, the pH was adjusted using 0.1N NaOH and/or 0.1M HCl and salinity was adjusted using reagent water or 5M NaCl (p. 19). The temperature of the water was monitored at each sampling interval.

The paint cylinders were sampled on days 1, 3, 7, 10, 14, 21, 24, 28, 31, 35, 38, 42, 45, 51, 58, 63, 70, 77, 84, and 91 (p. 19). Saltwater (1500 mL) was added to each of three labelled sample collection vessels placed into the waterbath of the dissolution system. The three paint cylinders for each treatment were removed from the holding tanks and attached to the sample collection stirrer, which was positioned over the sample collection vessels such that each paint cylinder was submerged in the saltwater of its corresponding sample collection vessel. The paint cylinders were spun for 1 hour at 60 rpm, then removed and placed back into their respective holding tank. The control sample was also sampled as described. After 91 days, the paint cylinders were removed, rinsed with water, and allowed to dry (p. 20). The thickness of the remaining paint was measured with a micrometer.

4. Supplementary Experiments:

A method validation test for zinc pyrithione was conducted to validate the recovery from substitute ocean water at fortification levels of 50.3 ng/mL and 101 ng/mL (pp. 17-18). Aliquots (3 x 125 or 250 µL) of a 4.02 µg/mL zinc pyrithione solution were brought to 10 mL with substitute ocean water. Aliquots (4 mL) were added to derivatizing agent (200 µL) and allowed to sit for 30 minutes. The samples were analyzed using HPLC.

A method validation test for Econeal® 028 and CL 322,250 was conducted to validate the recovery from substitute ocean water at fortification levels of 25.2-26.2 ng/mL and 50.4-52.4

ng/mL (p. 18). Aliquots (3 x 50 or 100 μ L) of a 5.24 μ g/mL Econe[®] 028 solution or a 5.04 μ g/mL CL 322,250 solution were brought to 10 mL with substitute ocean water. The Econe[®] 028 samples were allowed to sit overnight at room temperature to allow hydrolysis to CL 322,250. All samples were analyzed for CL 322,250 using LC-MS/MS.

C. Analytical Methods:

Prior to analysis, a derivatization of zinc pyrithione and Econe[®] 028 was performed (pp. 19-20). The derivatization reagent was prepared by mixing 5 mL of 2,2-dithiodipyridine solution (*ca.* 52 mg of 2,2-dithiodipyridine in 16 mL of acetonitrile and *ca.* 34 mL of reagent water) with 5 mL of disodium EDTA solution (*ca.* 200 mg of disodium EDTA in *ca.* 50 mL of reagent water; p. 12). Aliquots (100 μ L for zinc pyrithione; and 100 μ L for Econe[®] 028) of each spiking solution were brought to 10 mL with substitute ocean water and shaken with 500 μ L of freshly-prepared derivatization reagent. The samples were and allowed to stand at room temperature for at least 30 minutes, then analyzed by HPLC (zinc pyrithione) or LC-MS/MS (Econe[®] 028).

To analyze for zinc pyrithione, a portion of the saltwater in the sample collection vessels was transferred to 4 oz amber bottles (p. 19). An aliquot (10 mL) was transferred from the amber bottles to 14 mL amber vials. Derivatizing agent (500 μ L) was added to each vial for 30 minutes. The samples were analyzed for zinc pyrithione using HPLC under the following operating conditions (pp. 20-21): Phenomenex Aqua C18 column (4.6 mm x 250 mm; 5 μ m particle size), gradient mobile phase combining (Solvent System A) acetonitrile:water:phosphoric acid (25:75:0.1, v:v:v) and (Solvent System B) acetonitrile [percent A:B (v:v) at 0 min., 100:0; 15-20 min., 0:100; 20.1-26 min., 100:0], flow rate of 1.0 mL/minute with UV detection (234 nm). Zinc pyrithione was identified by comparison to chromatograms of a derivatized standard of zinc pyrithione. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for HPLC analysis were 1.41 ng/mL and 4.5 ng/mL, respectively (p. 26). Once per week, the water in the system was tested for the presence of zinc pyrithione by draining water from between the two carbon filters (p. 20). Samples (10 mL) were analyzed as previously described.

To analyze for Econe[®] 028, 10 mL of the saltwater in the sample collection vessels was transferred to 20 mL scintillation vials and allowed to sit overnight at room temperature (p. 20). The samples were analyzed for CL 322,250 using LC-MS/MS under the following operating conditions (pp. 21-22): MDS Sciex API 4000 LC-MS/MS with an Agilent 1100 series autosampler, column oven, and pump, Alltech Hypersil BDS C18 column (4.6 mm x 100 mm; 3 μ m particle size), gradient mobile phase combining (Solvent System A) 0.05% aqueous formic acid and (Solvent System B) methanol [percent A:B (v:v) at 0-1 min., 75:25; 4 min., 30:70; 6-9 min., 0:100; 9.5-13 min., 75:25], flow rate of 1.0 mL/minute with turbo ion spray in the negative mode. The LOD and LOQ for LC-MS/MS analysis were 0.157 ng/mL and 0.5 ng/mL, respectively (p. 26). Once per week, the water in the system was tested for the presence of Econe[®] 028 by draining water from between the two carbon filters (p. 20). Samples (10 mL) were analyzed as previously described.

The cumulative release rate ($R_{x,y}$, μ g/cm²) after each sample point was calculated using the following equation: $R_{x,y} = \Sigma[(R_i + R_j)/2](j-i)$, where R_i and R_j = the mean release rates

($\mu\text{g}/\text{cm}^2/\text{day}$) for each set of triplicate test cylinders for each pair of consecutive days from Day x through Day y, and i and j = time elapsed (days) since the start of the trial for each pair of consecutive data points (pp. 23-25). The mean release rate from day 21 to day 91 was calculated using the following equation: $\text{mean } R_{21,\text{end}} = \{\Sigma[(R_i + R_j)/2](j-i)\}/\Sigma(j-i)$, where R_i and R_j , as well as i and j are defined in the same way as described above. If the paint exhibited a pseudo-steady state, the pseudo-steady state release rate was calculated as described for the mean release rate.

II. RESULTS AND DISCUSSION

A. Test Conditions:

The study was conducted under laboratory conditions using synthetic ocean water. The average temperature of the system was $25.3 \pm 0.1^\circ\text{C}$ throughout the study (p. 27; Figure 8, p. 47). The turnover rates of the chambers ranged from 5.11 to 6.38 per hour. The pH of the system ranged from 7.86-8.08, and the salinity ranged from 30.4-35.1 ppt (Figures 6-7, pp. 45-46; Appendix II, p. 64). The thickness of the paint on the cylinders prior to study initiation was $>100\ \mu\text{m}$. At study termination, the thickness was $>50\ \mu\text{m}$ (p. 27; Table 4, p. 33; see Reviewer's Comment).

B. Anomalies:

None reported.

C. Summary:

For **zinc pyrithione**: The mean release rate from the painted cylinders from Day 21 to Day 91 was $8.27\ \mu\text{g}/\text{cm}^2/\text{day}$ (p. 27; Table 5, p. 34; Figure 13, p. 52). The cumulative release rate from the painted cylinders was $884\ \mu\text{g}/\text{cm}^2$ at Day 91 (Table 6, p. 35). The release rate reached a pseudo-steady state beginning with Day 58. The mean release rate over the pseudo-steady state period (Day 58 to Day 91) was $6.12\ \mu\text{g}/\text{cm}^2$ (Table 7, p. 36).

The calibration curve which was generated from the series of zinc pyrithione standards yielded correlation coefficients of ≥ 0.995 (p. 27; Figures 9-12, pp. 48-51). Spike recovery ranged from 92.2-103.5%. Zinc pyrithione was never detected in the re-circulation tank and system in the weekly test for breakthrough.

For **Econea® 028**: The mean release rate from the painted cylinders from Day 21 to Day 91 was $10.1\ \mu\text{g}/\text{cm}^2/\text{day}$ (p. 28; Table 8, p. 37; Figure 18, p. 57). The cumulative release rate from the painted cylinders was $912\ \mu\text{g}/\text{cm}^2$ at Day 91 (Table 9, p. 38). The release rate did not reach a pseudo-steady state (Table 10, p. 39).

The calibration curve which was generated from the series of zinc pyrithione standards yielded correlation coefficients of ≥ 0.995 (p. 27; Figures 14-17, pp. 53-56). Spike recovery ranged from 90.1-106.5% (p. 28). The CL 322,250 standards had recoveries ranging from 95.5-105.4%. Neither Econea® 028 nor CL 322,250 was ever detected in the re-circulation tank and system in the weekly test for breakthrough.

Supplementary Experiments: For zinc pyrithione, mean recoveries of the validation samples were $101.4 \pm 0.5\%$ and $99.2 \pm 0.7\%$ at nominal concentration levels of 50.3 and 101 ng/mL, respectively (p. 26; Table 1, p. 30; Figure 3, p. 42).

For Econeal® 028, mean recoveries of the validation samples were $97.2 \pm 1.2\%$ and $93.8 \pm 1.8\%$ at nominal concentration levels of 26.2 and 52.4 ng/mL, respectively (p. 26; Table 2, p. 31; Figure 4, p. 43). For CL 322,250, mean recoveries of the validation samples were $77.8 \pm 5.7\%$ and $86.3 \pm 2.5\%$ at nominal concentration levels of 25.2 and 50.4 ng/mL, respectively (Table 3, p. 32; Figure 5, p. 44).

III. STUDY DEFICIENCIES

The study author noted one deviation from ASTM Designation: D6442-05:

1. Deviations from the required salinity range of the synthetic ocean water (33-34 ppt) occurred at various times throughout the study (p. 28; Appendix II, p. 64).

The reviewer noted the following deviations from ASTM Designation: D6442-06:

1. The exposure surfaces of the test cylinders were prepared by lightly sanding with 400 grit sandpaper (p. 16). ASTM Designation: D6442-06 specifies that the test cylinders must be sanded with 200-grit sandpaper to provide a suitable surface for adhesion of the test paint (p. 3 of ASTM Designation: D6442-06).
2. It was not reported whether laboratory ware was properly cleaned/treated prior to use in the study. According to ASTM Designation: D6442-06, polycarbonate ware must be rinsed with deionized water, and all glass laboratory ware must be soaked in 10% HCl for a minimum of 6 hours or soaked in concentrated HCl for 30 minutes then rinsed with deionized water (p. 3 of ASTM Designation: D6442-06).
3. Following each application of formulated test paint, the relative humidity during the 7-day drying period was not reported. ASTM Designation: D6442-06 specifies that the painted cylinders must be dried at $25 \pm 2^\circ\text{C}$ and a relative humidity of 30-80% (p. 3 of ASTM Designation: D6442-06).
4. It was not reported whether the test cylinders were rotated during the definitive study. According to ASTM Designation: D6442-06, the test cylinders should be rotated at 60 ± 5 rpm (0.2 ± 0.02 m/s, velocity of test cylinder surface; p. 2 of ASTM Designation: D6442-06).

IV. REVIEWERS COMMENTS

1. The study was conducted according to ASTM methods D 5108-90 and D 6442-05. The reviewer critiqued the study report according to the ASTM Designation: D6442-06.
2. The length of the band of formulated test paint was not specified as 10 cm, as required in ASTM Designation: D6442-06; however the study author did report that the painted area was 200 cm², as required in ASTM Designation: D6442-06.
3. The study was initiated on December 26, 2005 and completed on March 29, 2006 (p. 19).
4. The paint thickness at the start of the study was reported by the study author as >100 µm, and at study termination as >50 µm (p. 27). However, according to the data in Table 4, p. 33, the paint thickness in samples A1, A2, A3, and A4 at the start of the study was 135, 134, 144, and 179 µm, respectively, and the paint thickness at study termination was 168, 125, 154, and not analyzed, respectively. It is uncertain why there is a discrepancy in paint thickness descriptions reported by the study author. It is also uncertain why the paint thickness for some samples appears to be greater at the end of the study compared to the beginning. These data would indicate the possibility of some error in the paint thickness measurement methods.

V. REFERENCES

ASTM. 2006. Standard Test Method for Determination of Copper Release Rate from Antifouling Coatings in Substitute Ocean Water. D6442-06. Annual Book of ASTM Standards.
